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TITLE: Multivalent peptidomimetic conjugates as inhibitors of androgen receptor function in therapy-resistant prostate cancer

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Multivalent Peptidomimetic Conjugates as Inhibitors of Androgen Receptor Function in Therapy Resistant Prostate Cancer

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**14. ABSTRACT**

Androgens are hormones that play a critical role in stimulating prostate cancer growth. Androgens activate a protein called the androgen receptor (AR), which regulates genes involved in cell growth. Although powerful anti-androgen drugs can be administered to block AR action and have been used successfully to treat patients with prostate cancer, over time the tumors become resistant to the drugs, leaving few treatment options. The goal of this proposal is to develop a new approach to block AR activity and stop prostate cancer growth using a new family of molecules called multivalent peptidomimetic conjugates. To accomplish our goals, we will create a set of conjugates with anti-androgens linked to the peptidomimetic backbone at variable intervals along the molecular chain. We will test these molecules for their ability to bind to AR. Those that bind tightly will then be tested in tumor models to evaluate if they block androgen-dependent prostate cancer cell growth. To understand how these molecules block AR function, we will determine the three-dimensional structure of AR bound to the peptidomimetic conjugates. These studies will be used to guide our ability to tailor the conjugates for optimal interactions with the AR.

**15. SUBJECT TERMS**

androgen receptor, prostate cancer, peptidomimetic conjugates,

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## 1. INTRODUCTION:

Androgens are hormones that play a critical role in stimulating prostate cancer growth. Androgens activate a protein called the androgen receptor (AR), which regulates genes involved in cell growth. Although powerful anti-androgen drugs can be administered to block AR action and have been used successfully to treat patients with prostate cancer, over time the tumors become resistant to the drugs, leaving few treatment options. The goal of this proposal is to develop a new approach to block AR activity and stop prostate cancer growth using a new family of molecules called multivalent peptidomimetic conjugates. We have previously demonstrated that a conjugate with two ethisterone steroidal groups arrayed with eight intervening monomers, (MPC6), had the greatest anti-proliferative effect in LNCaP-abl cells (a cellular model advanced disease that express AR, and proliferates in the absence of androgen), with more or less intervening monomers in between the ethisterone ligands diminishing compound activities. We are now creating a set of MPC6 variants, and evaluating if they block androgen-dependent prostate cancer cell growth. To understand how these molecule blocks AR function, we will determine the three-dimensional structure of AR bound to MPC6. These studies will be used to guide our ability to tailor the conjugates for optimal interactions with the AR.

## 2. KEYWORDS: Androgen receptor, prostate cancer, peptidomimetic conjugates,

## 3. ACCOMPLISHMENTS:

- What were the major goals and objectives of the project?

**The major goals of the project are:**

1) Synthesize a family of multivalent peptidomimetic conjugates
2) Test peptidomimetic conjugates in a series of in vitro and cell-based assays
3) Conduct studies of pharmacological potential through *in vivo* mouse xenograft and PK/PD studies
4) Establish the mechanism of action of peptidomimetic conjugates on the Androgen Receptor through biophysical and X-ray crystallographic studies.

- What was accomplished under these goals? Major activities for this reporting period:

- **Major Task 1:** Synthesize a family of multivalent peptidomimetic conjugates
- Subtask 1: Design, synthesize, purify and characterize a family of peptidomimetic conjugates

We synthetized a set of MPC6 variants with increase linker length, an alternative linker to enhance solubility and one with a different linkage to impart distinct structural characteristics (Fig.1). We will test these in *in vitro* and cell based assays.

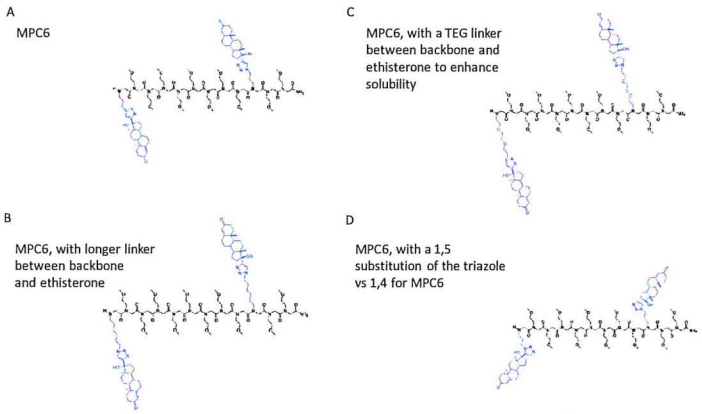


**Figure 1. MPC6 variants**
A) Shown are the chemical structures of MPC6 variants. A) MPC6; B) MPC6 with a longer linker between the backbone and ethisterone, C) MPC6 with an alternative linker with the potential to enhance solubility in aqueous solutions, D) MPC6 with ethisterone linked at a different position on the backbone. 1,5 substitutions of the triazole linkage vs 1,4 for MPC6.

**Major Task 2:** Test the peptidomimetic conjugates in a series of in vitro and cell-based assays

Subtask 2: Conduct *in vitro* activity assays

- Determine peptidomimetic conjugates ability to compete for DHT-binding to the AR LBD in vitro
- Determine peptidomimetic conjugates impact on coactivator peptide binding in vitro

*In progress:*

Subtask 3: Conduct cell-based activity assays

- Examine the ability of the peptidomimetic conjugates to modulate AR-dependent transcriptional activity
- Determine peptidomimetic conjugates ability to promote AR-YFP nuclear localization and to block DHT-dependent AR nuclear localization
- Validate the impact of peptidomimetic conjugates on AR transcriptional activation of endogenous AR target genes by qPCR, and recruitment of AR to targets by ChIP
- Evaluate peptidomimetic conjugates ability to inhibit proliferation of therapy-resistant prostate cancer cells

MPC6 exhibited anti-proliferative effects against enzalutamide-resistant LNCaP-abl cells (Fig 2A) and LNCaP-95 cells, but not AR-deficient PC3 prostate cancer cells or HEK293 cells(Wang, Dehigaspitiya et al. 2016). MPC6 also reduced colony formation from single cells in a clonogenic assay (Fig 2B), whereas enzalutamide and ARN509, a competitive inhibitor of AR mechanistically similar to enzalutamide but with greater efficacy in xenograft models, failed to do so. MPC6 also exhibited favorable pharmacologic properties, with low clearance rate in both human and rat liver microsomes, low plasma-binding properties, and high bioavailablity via both intraperitoneal (IP) and intravenous routes. In addition, MPC6 displayed *in vivo* efficacy in LNCaP-abl cells (Fig 2C) and LNCaP-95 (not shown) xenografts (Wang, Dehigaspitiya et al. 2016). After 3.5 weeks of twice-a-week treatment at 50 mg/kg via IP administration, MPC6 reduced but did not eliminate tumor growth, suggesting that potency could be improved. Thus, ethisterone peptoid conjugates have the potential to reduce prostate tumor growth *in vivo* in an immunocompromised mouse model with moderate potency.

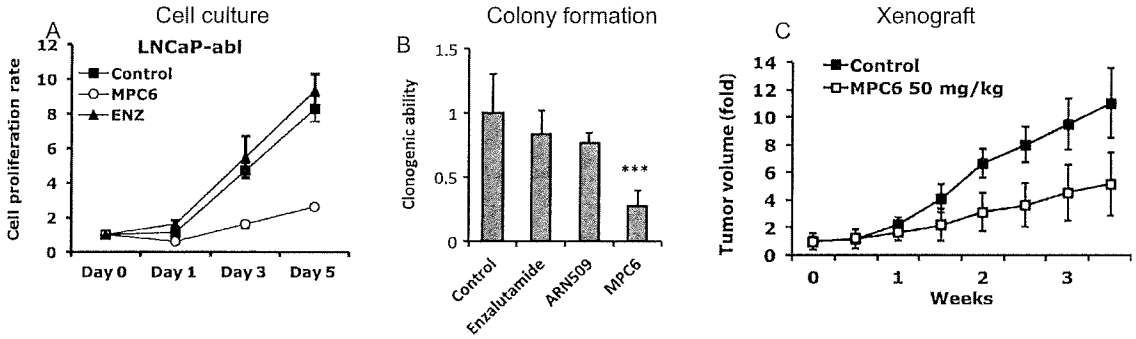


**Figure 2 Ethisterone-peptoid conjugate MPC6 suppresses LNCaP-abl growth *in vitro* and *in vivo*.** A) Enzalutamide-resistant LNCaP-abl cells were treated with vehicle (control), MPC6 (10 uM), or enzalutamide (10 uM) for 5 days, and cell growth was measured. B) Colony formation in LNCaP-abl cells treated with vehicle, MPC6 (10 uM), enzalutamide (10 uM), or AR509 (10uM) was measured by a clonogenic assay. ***, P = 0.0003. C) Nude mice (Nu/J) bearing LNCaP-abl xenografts were treated with DMSO vehicle (n =10) or 50 mg/kg MPC6 (n =10) twice a week for 3.5 weeks. Tumor volumes were measured on the indicated days (P = 0.004).

**Major Task 4.** X-ray crystallography of the AR-MPC6 complex. The AR ligand binding domain (LBD) remains a very challenging crystallization target because it expresses poorly. In order to obtain enough protein for crystallization trials, we grew > 24 liters of E Coli, and purified it to homogeneity (Fig. 3A). However, AR can only be grown in the presence of an agonist, which was required to stabilize the protein. With the MPC6 antagonist, there was no expression (Fig. 3B). This is a common feature of the related steroid receptors, MR, GR, and PR, which have been very difficult to purify with antagonists. With the estrogen receptor, we have been successful in exchanging ligands. After several rounds of dialysis with MPC6, we set up extensive crystallization trials and obtained crystals, but unfortunately the electron density very clearly showed that the bound ligand was in fact the dihydroxytestosterone (DHT) that was used to grow the protein in E. coli (Fig. 3C).

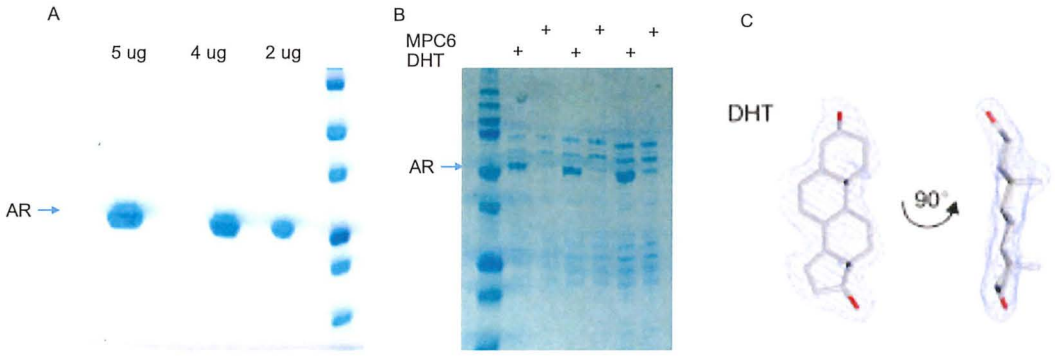


**Figure 3 Purification of AR.** A) Twenty-four liters of E. coli were grown with 50uM DHT and purified with nickel affinity resin, ion exchange, and gel filtration. Purity is shown by SDS PAGE gel. B) AR expression test. AR was expressed in E coli with either DHT or MPC6 and purified by Nickel affinity beads. Only DHT stabilized AR protein. C) X-ray crystal structure of AR. AR was grown and purified with DHT, and then subject to multiple rounds of dialysis of MPC6. Structures of 8 different crystals revealed electron density for the ligand that was clearly DHT. Shown is the 2F-Fo map surrounding the ligand at 1 sigma for one of the structures.

- **What opportunities for training and professional development did the project provide?**
  Nothing to Report."

- **How were the results disseminated to communities of interest?**
  We published a paper on this topic in Cancer Research Cancer Res. 2016 Sep 1;76(17):5124-32. doi: 10.1158/0008-5472.CAN-16-0385. Epub 2016 Aug 3, which is widely read by basic and clinical oncologists. The study was also highlighted in the journal *Nature Reviews Urology*, which targets Urologists in clinical practice.

- **What do you plan to do during the next reporting period to accomplish the goals and objectives?**

  We are planning to continue to synthesize and test additional derivatives of MPC6 that include cationic side chains to improve efficacy. We will test all of the MPC6 variants in the *in vitro* and cell based assays. We are waiting to test all of the compounds simultaneously to minimize experimental variability. For the most potent derivatives we also plan DMPK and xenograft studies to determine the impact on therapy resistant prostate cancer cell (LNCaP-abl and LNCaP-95) *in vivo*.

  With respect to the challenging crystallography task, we are pursuing several alternate strategies: 1) we are developing protocols for purification of a larger fragment of AR containing both ligand and DNA binding domains, to test if this construct will be more stable during

expression; 2) we will try extended dialysis for up to 7-14 days; 3) we will use a method recently described to us by Jim Keifer, Director of Structure Biology at Genentech, to soak ligands into recalcitrant proteins. This involved transferring crystals of the DHT-bound AR into a large volume of very high concentration MCP6 (100-200 ul of 100mM stock) in the crystallization solution.

## 4. IMPACT:

Our work describes the biological evaluation of a new set of multivalent peptoid conjugates (MPCs) We are discovering the therapeutic potential of an innovative new family of compounds against late-stage prostate cancer that is resistant to current treatments.

**What was the impact on technology transfer?**

We are continuing to work with NYU's Office of Therapeutics Alliances to develop a patent portfolio covering the technologies relevant to multivalent conjugates targeting the Androgen Receptor.

**What was the impact on society beyond science and technology?**

Nothing to report

## 5. CHANGES/PROBLEMS:

Nothing to Report

## 6. PRODUCTS:

Wang Y, Dehigaspitiya DC, Levine PM, Profit AA, Haugbro M, Imberg-Kazdan K, Logan SK, Kirshenbaum K, Garabedian MJ. Multivalent Peptoid Conjugates Which Overcome Enzalutamide Resistance in Prostate Cancer Cells. Cancer Res. Cancer Res. 2016 Sep 1;76(17):5124-32. doi: 10.1158/0008-5472.CAN-16-0385. Epub 2016 Aug 3.

Thomas, C., Fitting to overcome enzalutamide resistance, *Nature Reviews Urology* (2016) doi:10.1038/nrurol.2016.160 Published online 23 August 2016

## 7: PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

PIs: Name: Kent Kirshenbaum, PhD
Project Role: Initiating PI.
Nearest person month worked: 1
Contribution to Project:
Dr. Kirshenbaum conceived of the chemical platform and helped with the design and synthesis of the peptoid conjugates. He analyzed the experiments involving the chemical synthesis, and the cellular studies of MPC6 and its derivatives. He assisted in authoring the recent paper on MPC6 function and activity in prostate cancer
Funding Support: CDMRP

Name: Michael Garabedian, PhD.
Project Role: Partnering PI.
Nearest person month worked: 3
Contribution to Project:
Dr. Garabedian helped design and analyze the experiments involving the cell based proliferation assays and in vitro biochemical studies on MPC6 on AR. He also helped write the paper on MPC6 function and activity in prostate cancer.
Funding Support: CDMRP

Name: Kendall Nettles, PhD.
Project Role: Partnering PI.
Nearest person month worked: 3
Contribution to Project:
Dr. Nettles is performing the biophysical studies on the AR and MPC6. He is also involved in the DMPK studies.
Funding Support: CDMRP

Post docs and students

Name: Yu Wang, PhD
Project Role: Post doc
Nearest person month worked: 12
Contribution to Project:
Dr. Wang performed the experiments involving the cell based proliferation assays and in vitro biochemical studies on MPC6.
Funding Support: Prostate Cancer Foundation Young Investigator Awardee

Name: Amanda Kapser, PhD
Project Role: Post doc
Nearest person months worked: 6
Contribution to Project:
Dr. Kapser performed the synthesis of MPC6 and its derivatives.
Funding Support: NSF

Name: Dilani C. Dehigaspitiya, PhD
Project Role: Post doc
Nearest person months worked: 3
Contribution to Project:
Dr. Dehigaspitiya performed the synthesis of MPC6.
Funding Support: NSF

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
Nothing to Report

**What other organizations were involved as partners?**
Nothing to Report

**8 SPECIAL REPORTING REQUIREMENTS:**
**COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to https://ers.amedd.army.mil for each unique award.**

**9 APPENDICES:**
none

**Reference cited**

Wang, Y., D. C. Dehigaspitiya, P. M. Levine, A. A. Profit, M. Haugbro, K. Imberg-Kazdan, S. K. Logan, K. Kirshenbaum and M. J. Garabedian (2016). "Multivalent Peptoid Conjugates Which Overcome Enzalutamide Resistance in Prostate Cancer Cells." Cancer Res **76**(17): 5124-5132.